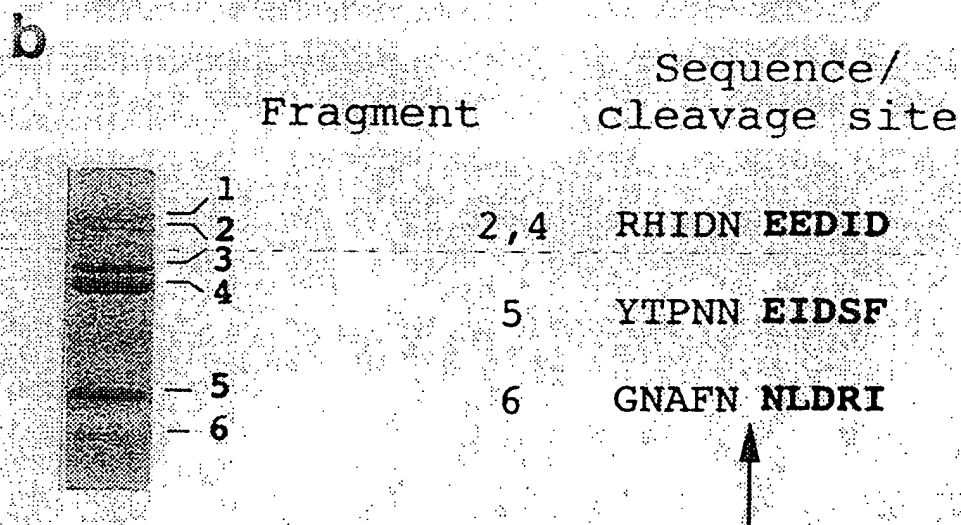
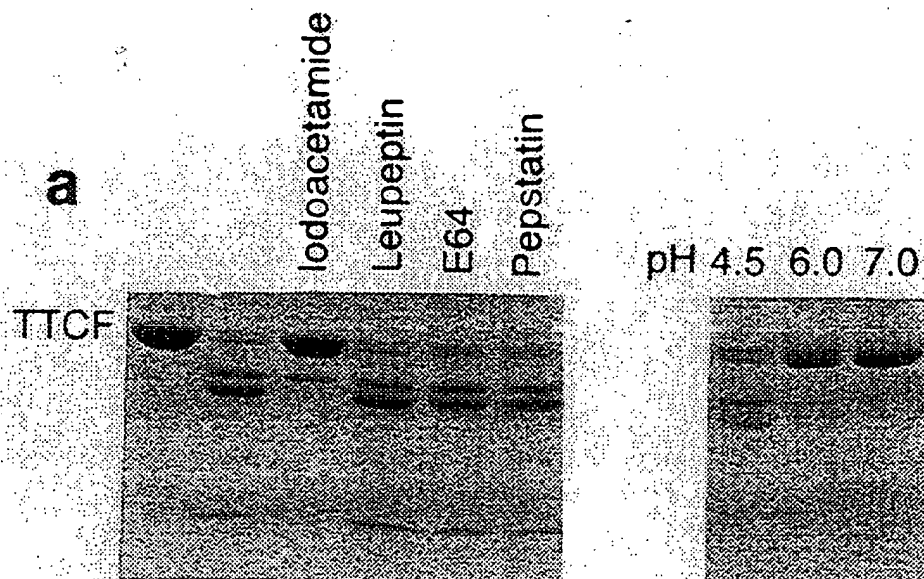
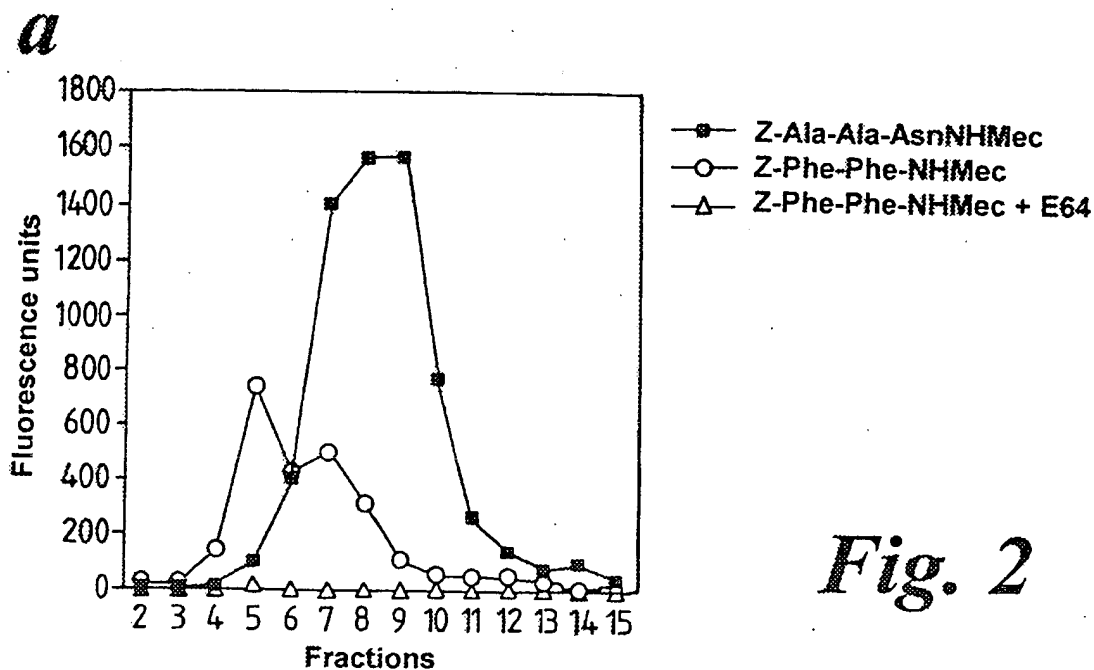
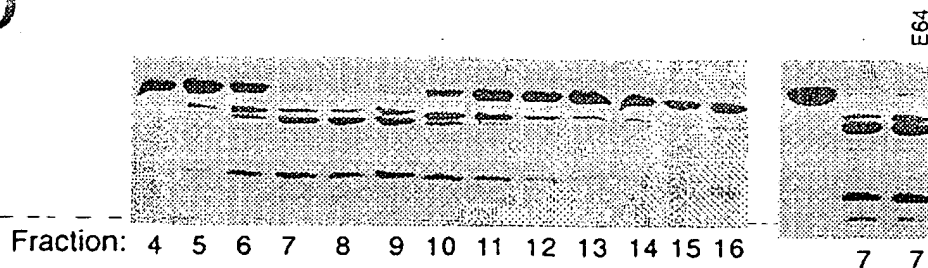


*Fig. 1 (page 2 of 2)*

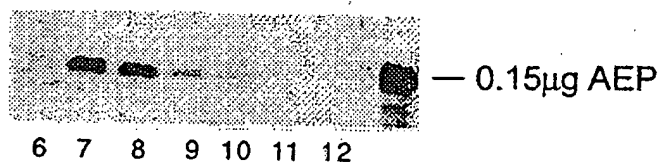
**Fig 1 (page 1 of 2)**

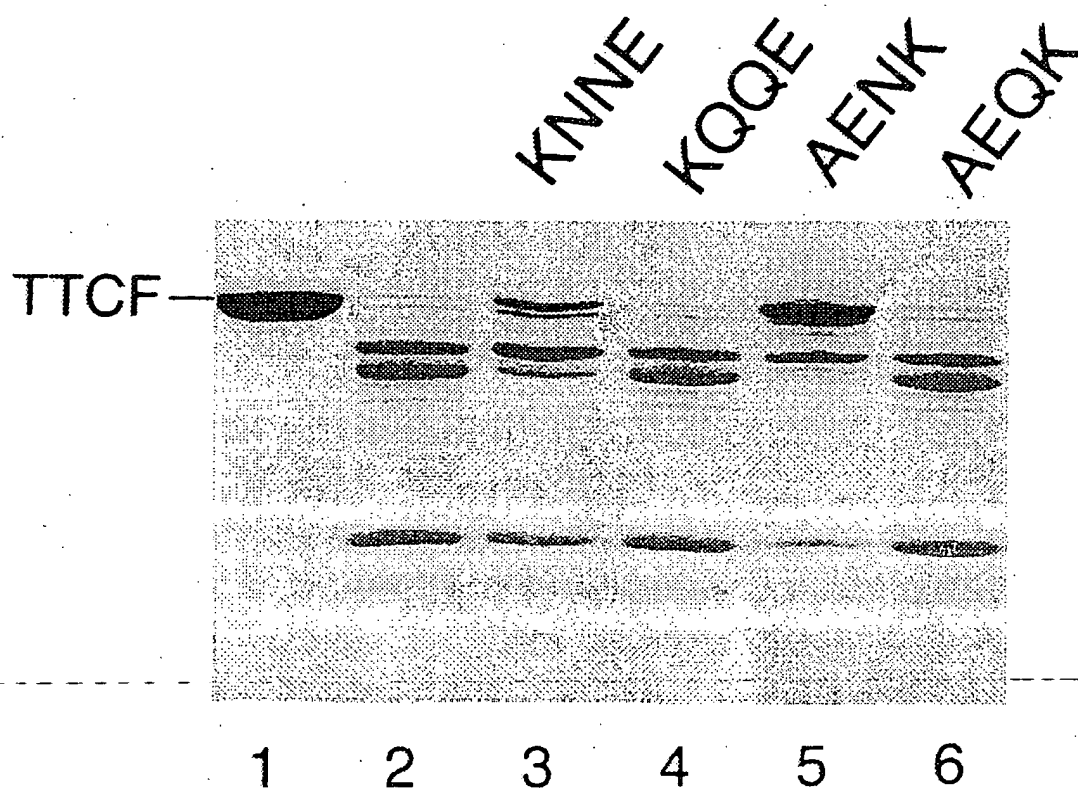
*Fig. 2*

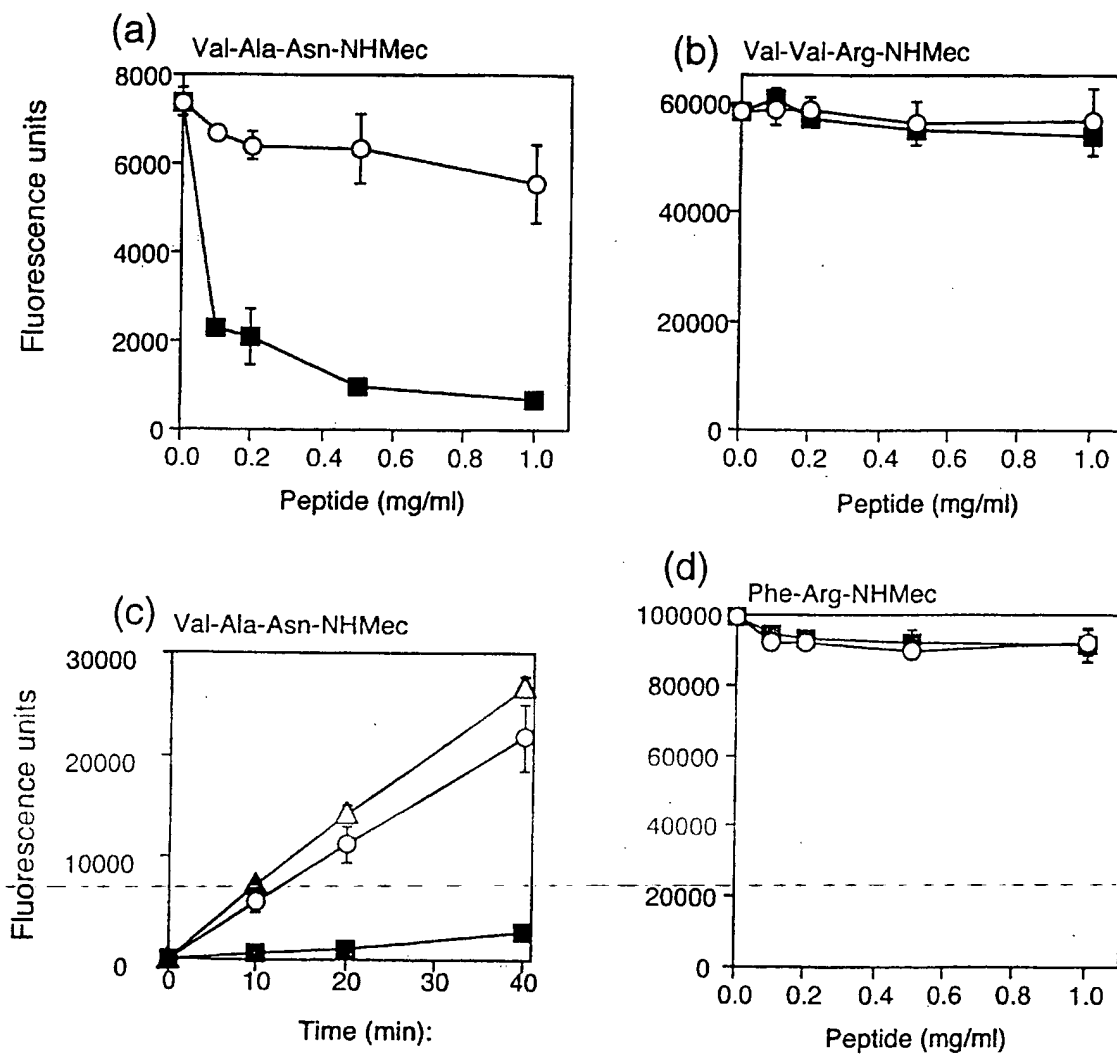
**b**

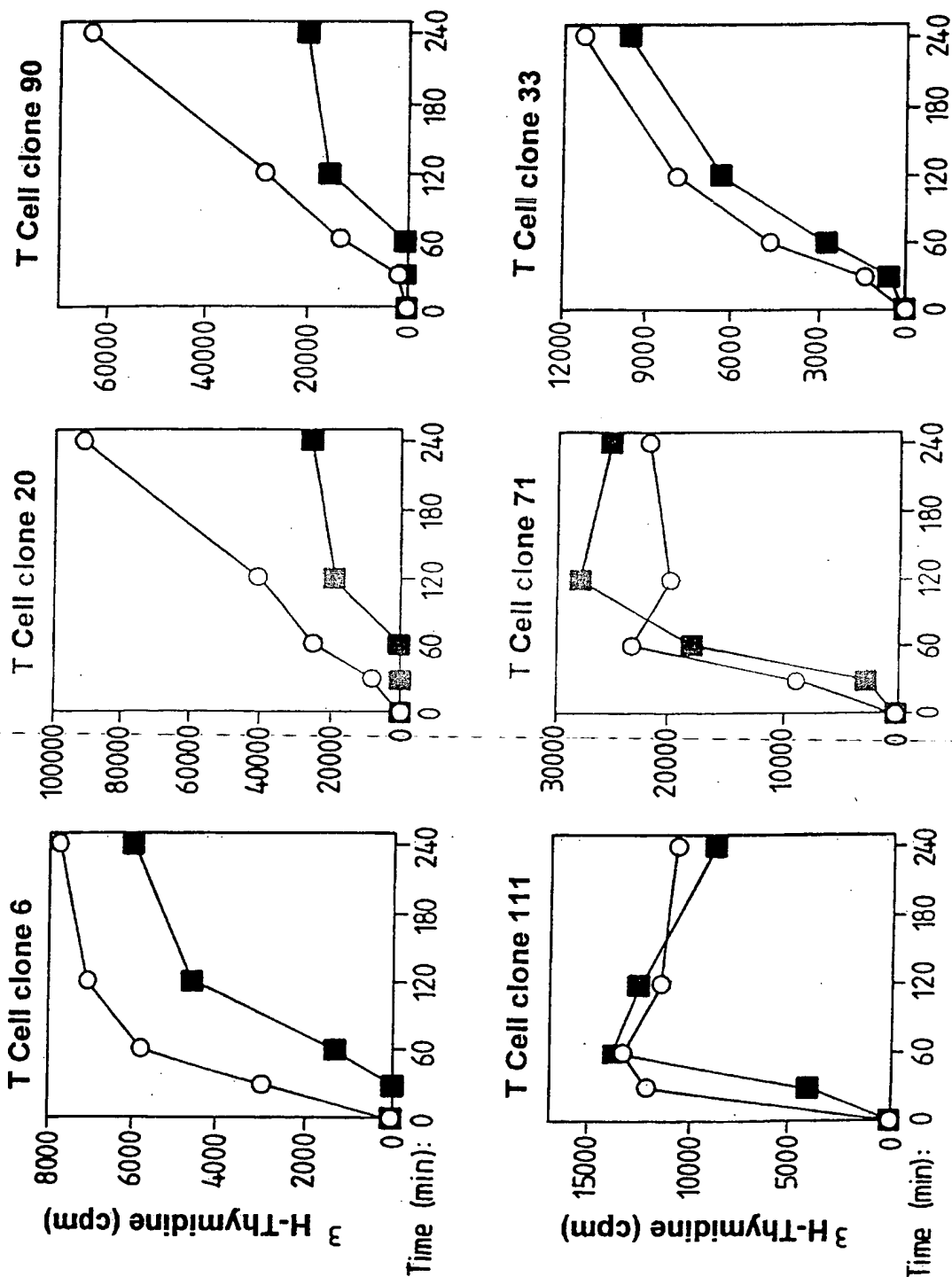


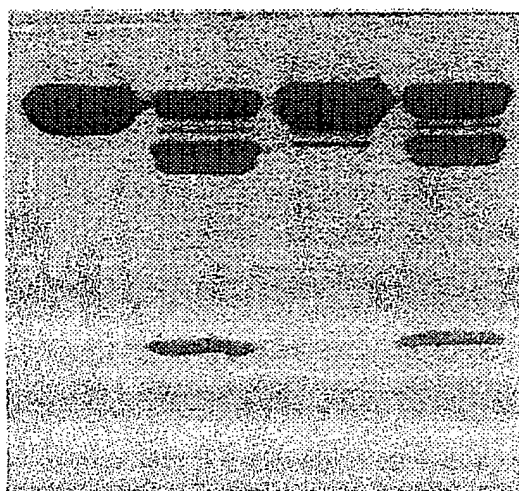
**c**



*Fig. 3a*

*Fig. 3b*

*Fig. 3c*

*Fig. 3d*AENK  
AEQK

— + + +

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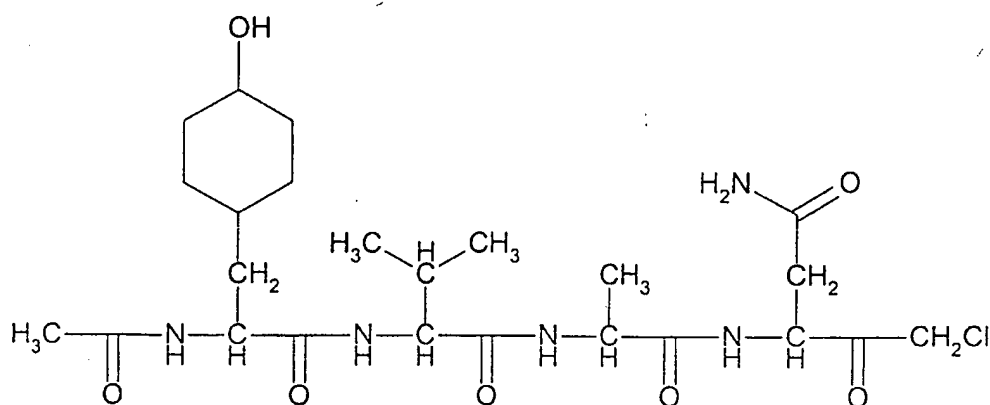
Human B cell AEP  
 $\pm$  1.0 mg/ml peptide

7/24

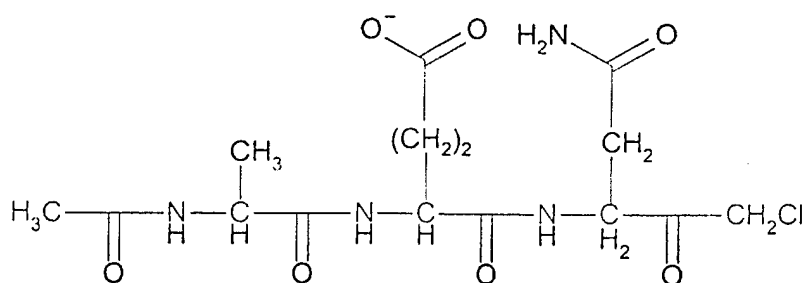
Figure 4 (page 1 of 6)

## Peptidyl chloromethylketones (ref 5)

- (i) Acetyl-tyrosyl-valyl-alanyl-asparaginyl-chloromethylketone  
(analogous to ICE protease inhibitor YVAD-cmk)

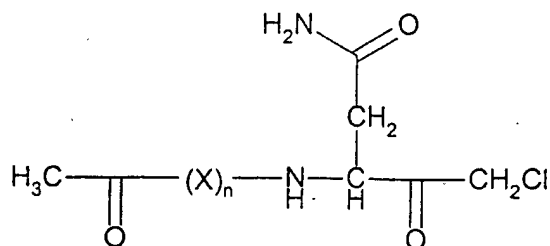


- (ii) Acetyl-alanyl-glutamyl-asparaginyl-chloromethylketone



- (iii) Acetyl (or benzyloxycarbonyl)-(X)<sub>n</sub>-asparaginyl-chloromethylketone

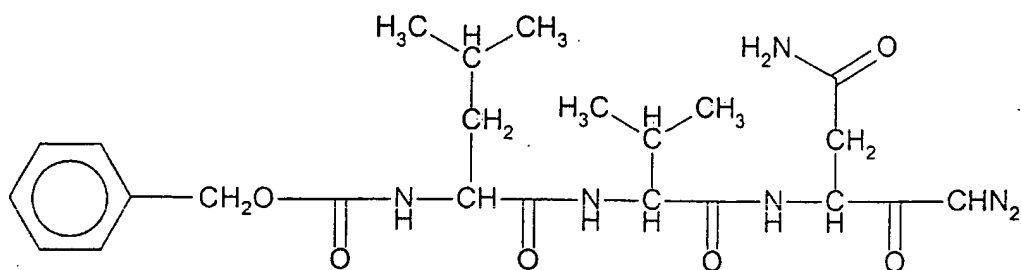
Where X = any amino acid



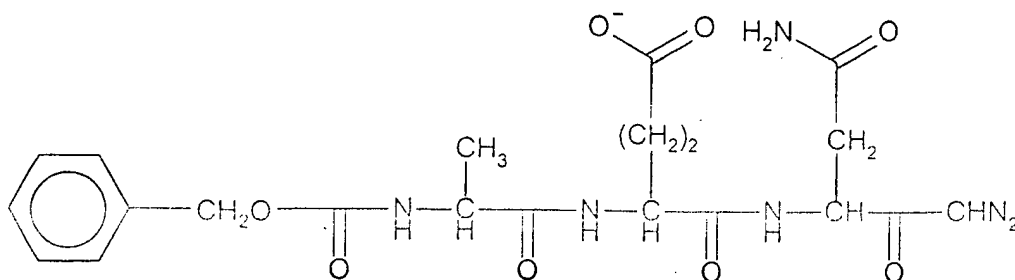
# Figure 4 (page 2 of 6)

Peptidyl diazomethanes (ref 3,4)  
(have the general structure:  $R-C(=O)CHN_2$ )

(i) Benzyloxycarbonyl-leucyl-valyl-asparaginyl-diazomethane

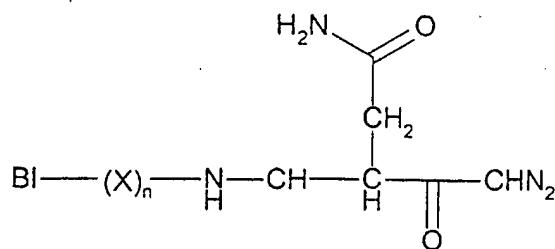


(ii) Alanyl-glutamyl-asparaginyl-diazomethane



(iii) Z-(X)<sub>n</sub>-asparaginyl-diazomethane

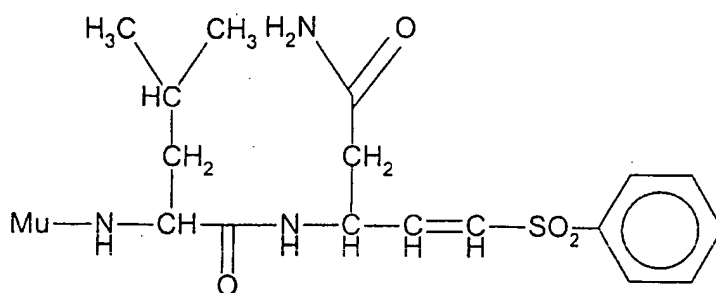
Where BI = acetyl or benzyloxycarbonyl and X = any amino acid



## Figure 4 (page 3 of 6)

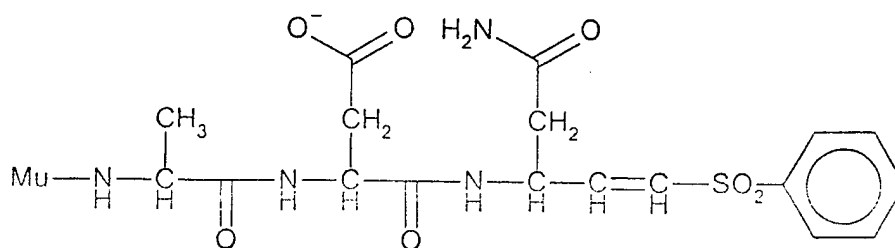
Peptidyl vinyl sulphones (ref 6)

- (i) Morpholinurea-leucyl-asparaginyl-vinylsulphone-phenyl  
(Acetyl or benzyloxycarbonyl can replace morpholinurea)



Mu - morpholinurea

- (ii) Morpholinurea-alanyl-glutamyl-asparaginyl-vinylsulphone-phenyl  
(Acetyl or benzyloxycarbonyl can replace morpholinurea)



- (iii) BI-(X)<sub>n</sub>-asparaginyl-vinylsulphone-R

Where BI = N-terminal blocking group (acetyl, morpholinurea or benzyloxycarbonyl), X = any amino acid and R = alkyl or aryl terminating group

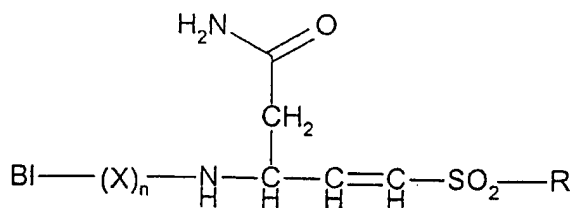
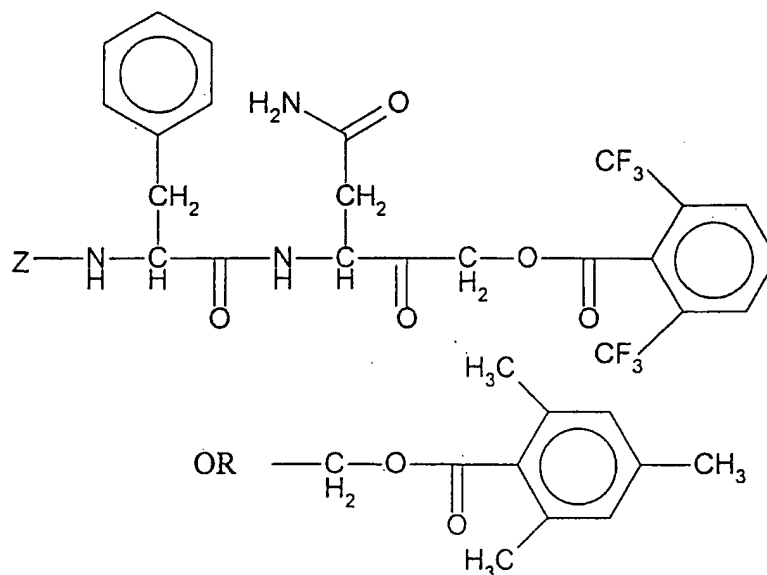


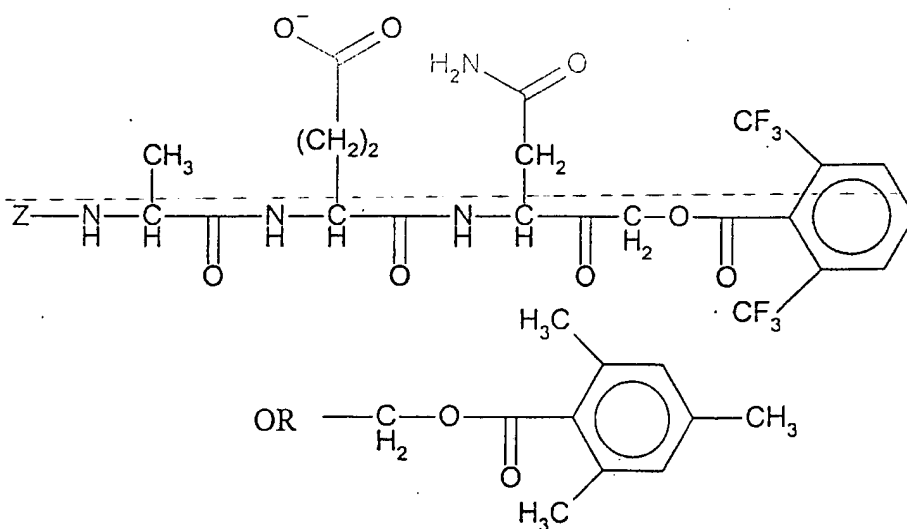
Figure 4 (page 4 of 6)

Peptidyl (acyloxy) methanes (ref 7)

- (i) Z-Phenylalanyl-asparaginyl-CH<sub>2</sub>OCO-[2,6-(CF<sub>3</sub>)<sub>2</sub>Phenyl]  
 (ii) Z-Phenylalanyl-asparaginyl-CH<sub>2</sub>OCO-[2,4,6-(CH<sub>3</sub>)<sub>3</sub> Phenyl]



- (iii) Z-alanyl-glutamyl-asparaginyl-CH<sub>2</sub>OCO-[2,6-(CF<sub>3</sub>)<sub>2</sub>Phenyl]  
 (iv) Z-alanyl-glutamyl-asparaginyl-CH<sub>2</sub>OCO-[2,4,6-(CH<sub>3</sub>)<sub>3</sub> Phenyl]

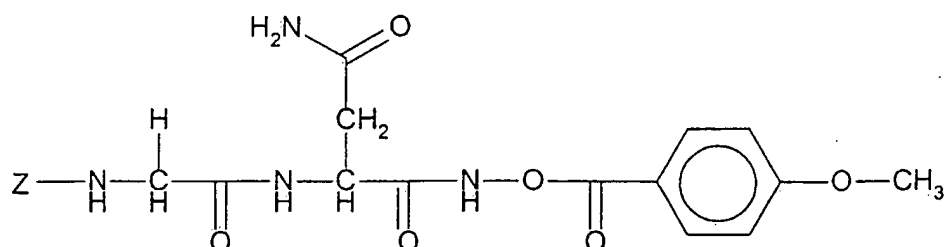


- (v) Z-(X)<sub>n</sub>-asparaginyl-CH<sub>2</sub>OCO-R

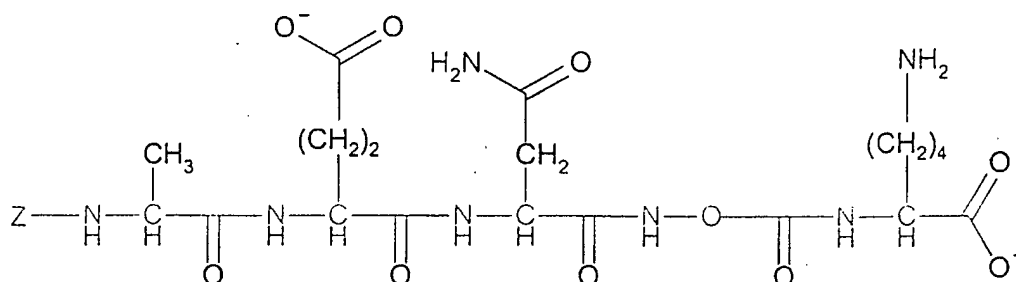
Where X = any amino acid and R = [2,6-(CF<sub>3</sub>)<sub>2</sub>Phenyl] or [2,4,6-(CH<sub>3</sub>)<sub>3</sub> Phenyl] or other acyloxy methane group

Figure 4 (page 5 of 6)

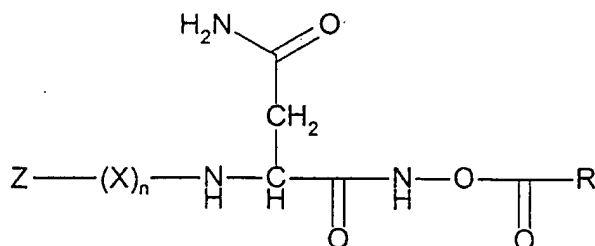
N,O-diacyl hydroxamates (ref 8)

(i) Z-Glycyl-asparaginyl-NHO-benzoyl(4-OCH<sub>3</sub>)

(ii) Z-alanyl-glutamyl-asparaginyl-NHO-CO-lysine-NH

(iv) Z-(X)<sub>n</sub>-asparaginyl-NHO-CO-R

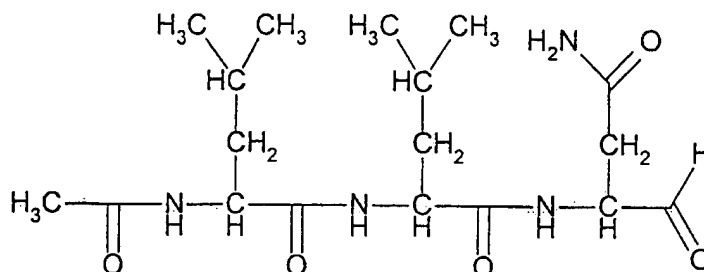
Where Z = benzyloxycarbonyl or other blocking group,  
X = any amino acid and R = any O-acyl group



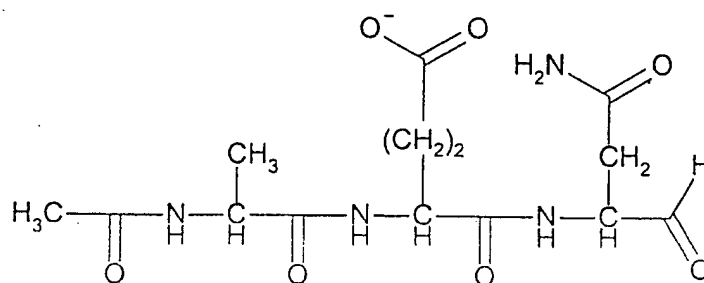
**Figure 4 (page 6 of 6)**

Peptide aldehydes (refs 1 &amp; 2)

(i) Acetyl-leucyl-leucyl-asparaginal



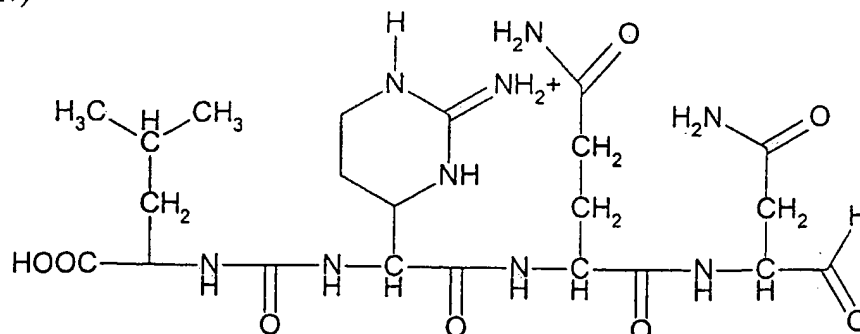
(ii) Acetyl-alanyl-glutamyl-asparaginal

(iv) Acetyl (or other blocking group)-(X)<sub>n</sub>-Asparaginal

-where X denotes any amino acid(s) in peptide linkage

Elastinal also blocks AEP. A more specific variant would be:

(iv)



09/646950

09/646950

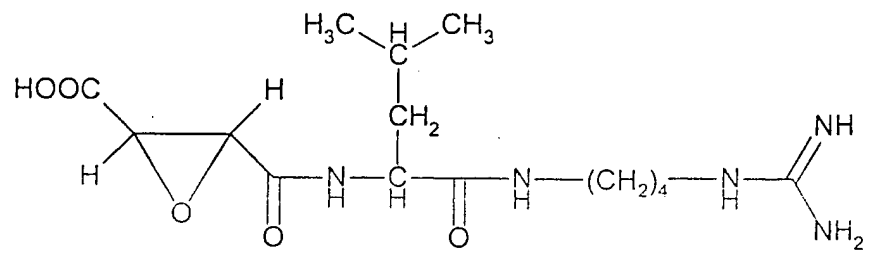
WO 99/48910

PCT/GB99/00963

Figure 5 (page 1 of 6)

E-64

Structure: L-trans-epoxysuccinyl-leucylamide-(4-guanido)-butane or  
N-[N-(L-trans-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatine



## Figure 5 (page 2 of 6)

## Leupeptin

Structure: Acetyl-leucyl-leucyl arginal

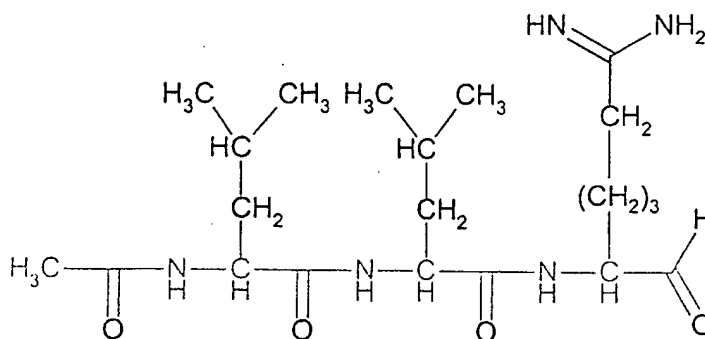
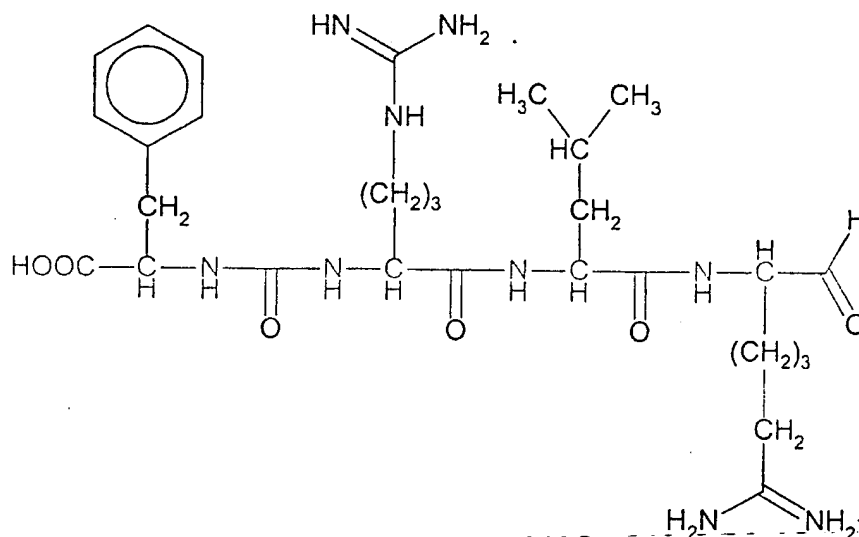


Figure 5 (page 3 of 6)

## Antipain

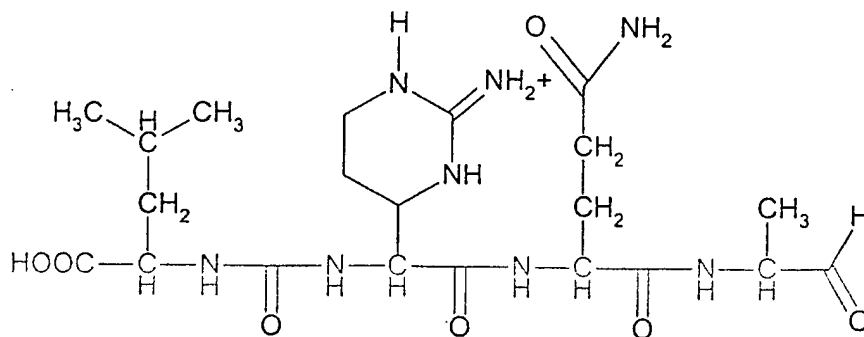
Structure: [(S)-1-Carboxy-2-Phenyl]-carbamoyl-Arg-Val-arginal



## Figure 5 (page 4 of 6)

## Elastinal

Structure: Leu-(Cap)-Gln-Ala-al,  
N-[(S)-1carboxy-isopentyl]-carbamoyl-alpha-(2-iminohexahydro-4(S)-  
pyrimidyl)-L-glycyl-L-glutaminyl-L-alaninal



## Figure 5 (page 5 of 6)

## TLCK

Structure: Tosyl Lysyl ChloromethylKetone:  
1-Chloro-3-tosylamido-7-amino-2-heptanone

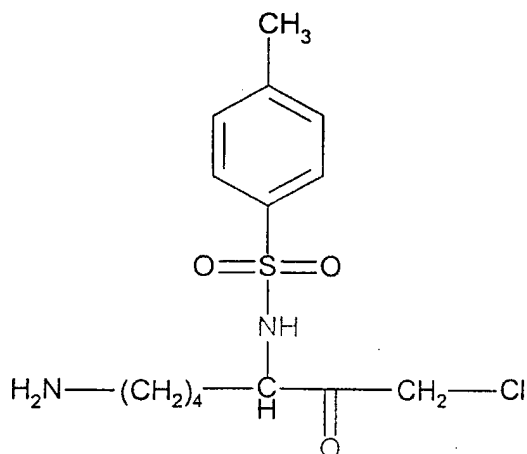
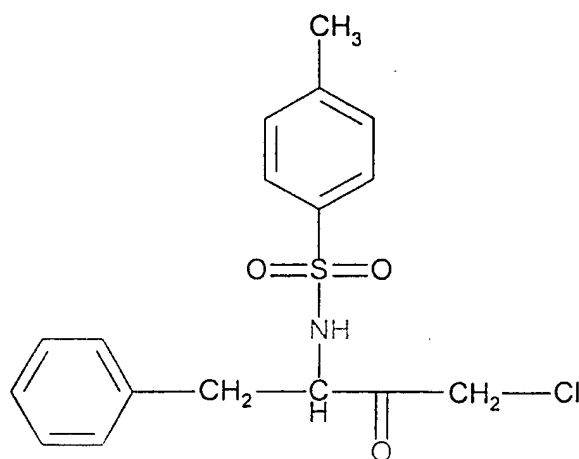


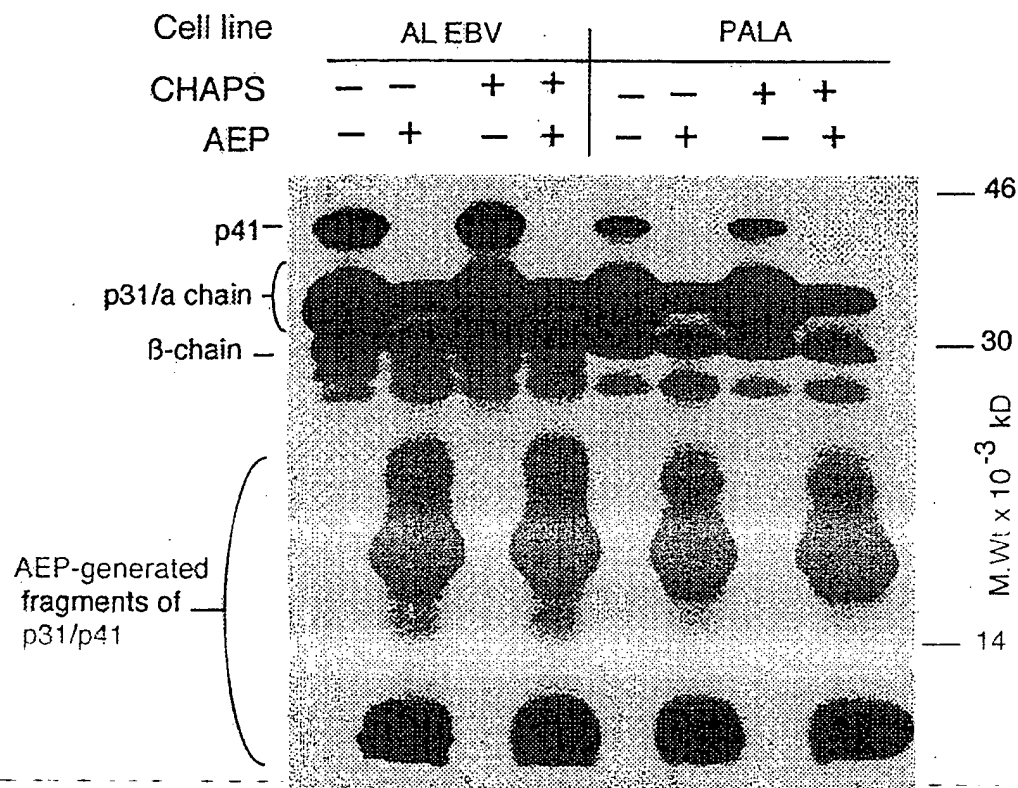
Figure 5 (page 6 of 6)

## TPCK

Structure: Tosyl Phenylalanyl ChloromethylKetone:  
1-Chloro-3-tosylamido-4-phenyl-2-butanone



**Fig. 6**

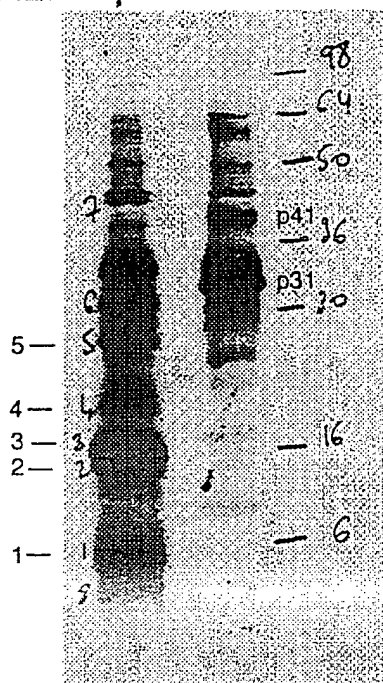


# AEP processing of the invariant chain

(a)

VIC  $\gamma$ 1 precipitate

AEP + -

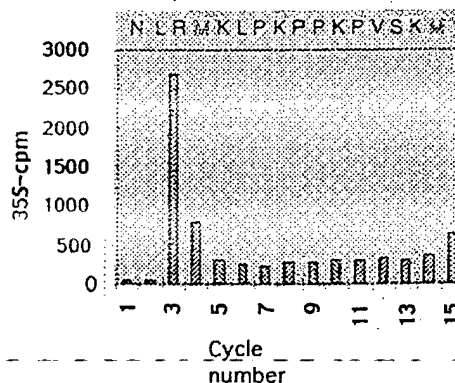


**Fig. 7**

(b) N-terminal sequence of bands below

- Band 1: N NEQLPM (Peak cycle 5/6)
- Band 2: N TMETI (Peak cycle 2)
- Band 3: N LRMK (Peak cycle 3)
- Band 4: N LRMK  
N NEQLPM (Peaks at cycles 3 & 6)
- Band 5: N LRMK

Example raw data from band 5



(c)

MDDQ<sup>↓</sup>RDLISNNEQLPMLGR<sup>↓</sup>RPGAPESKCSR<sup>↓</sup>GALYTGFSILVTLLLAGQATTAYF  
 QQQGR<sup>↓</sup>LDKLT<sup>↓</sup>VT<sup>↓</sup>TSQNLQLENLRMKLPKPPKPVSKMRMATPLL<sup>↓</sup>NOALPMGALPQG  
 QNATKYGNMTEDHVMHLLQNADPLKVYPPLKGSFPENLTHLKNTMETIDWKVFE  
 MHHWLLFEMSRHSLEQKPTDAPPKESLELEDPSGLGVTKQDLGPVPM

5017

# Fig 8

## Identified AEP cleavage sites

### Tetanus toxin C fragment

RHIDN EEDID  
YTPNN EIDSF  
GNAFN NLDRI

### Ribonuclease

NGQTN CYQSY  
VACKN GQTNC

### Ovalbumin

GTSVN VHSSL

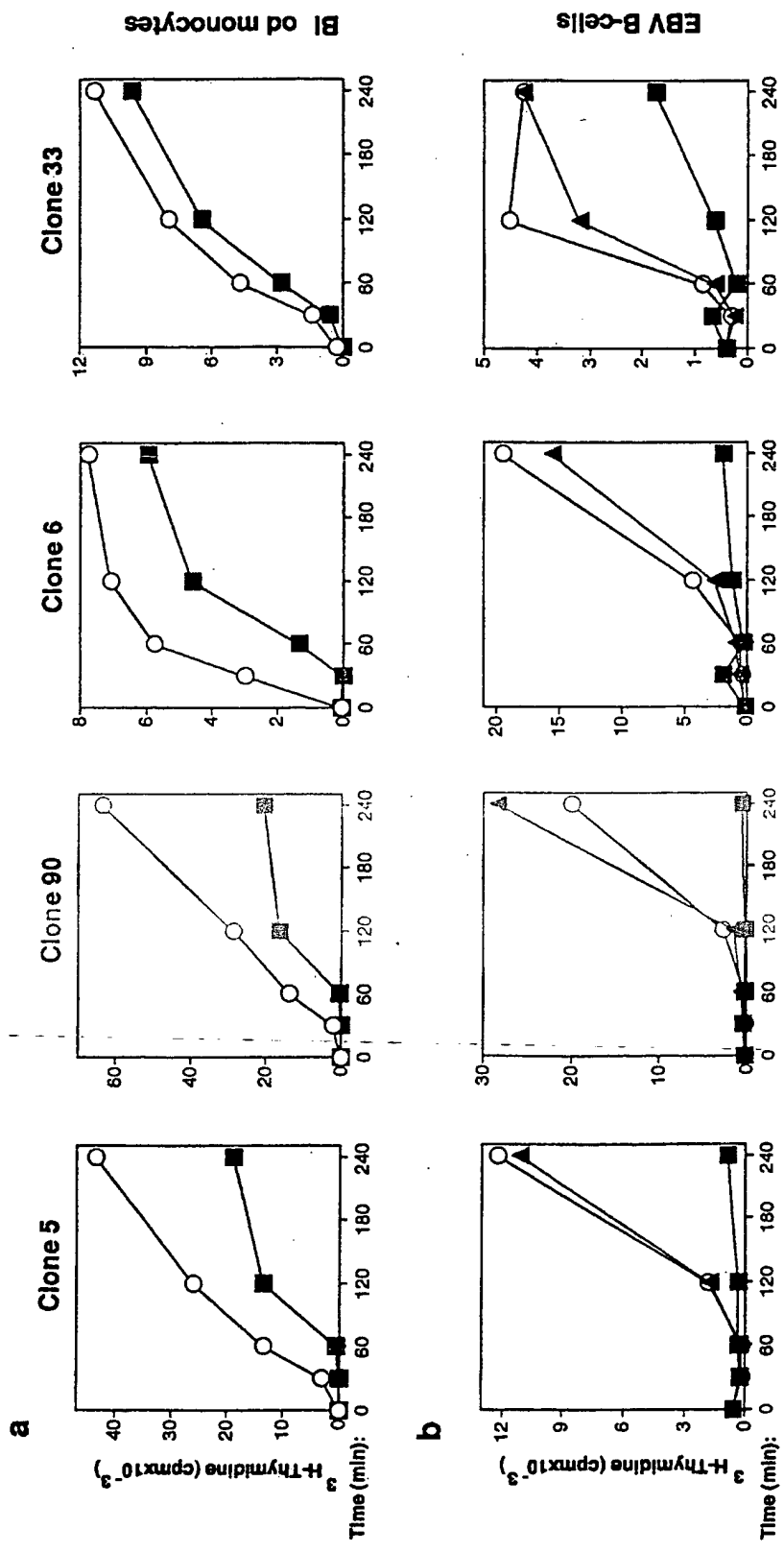
### Hen Egg Lysozyme (preferred sites listed first)

GNGMN AWVAW  
HGLDN YRGYS  
ILQIN SRWWC  
VSDGN GMNAW  
RWWCN DGRTP  
VAWRN RCKGT

### Transferrin peptide (622-642)

LFGSN VTDCS  
DCSGN FCLFR

Fig. 9 (page 1 of 2)



# Fig 9 (page 2 of 2)

